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Short Communication

Biodiversity in seagrass meadows in contrast to adjacent bare sand habitats of Foros Bay (Bulgarian Black Sea coast) assessed using environmental DNA (eDNA) metabarcoding

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Abstract

The present study presents an initial eDNA-based assessment of metazoan biodiversity associated with *Zostera* seagrass meadows and neighbouring bare sand habitats in Foros Bay, located along the Bulgarian Black Sea coast. Environmental DNA metabarcoding targeting the 12S and 18S rRNA regions revealed species-specific habitat associations, the presence of economically important fish species and increased detection frequencies of benthic metazoans in seagrass habitats relative to bare sand. On the contrary, data analyses conducted at the community level revealed that the degrees of compositional heterogeneity, total fish detection and eDNA signals were comparable amongst habitats. This indicates that mobile taxa are not closely associated with particular habitat and that habitat effects depend on the ecological characteristics of the organisms and the spatial scale of the research. These results highlight that *Zostera* meadows can enhance species richness and diversity; however, they do not consistently stabilise community composition. Our findings, demonstrate that eDNA metabarcoding is an effective instrument for evaluating the ecological and management significance of coastal habitat mosaics.

Key words: Environmental DNA (eDNA), metabarcoding, metazoan, seagrass

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Introduction

Seagrass meadows are highly productive ecosystems and functional biodiversity hotspots in the coastal areas (Waters et al. 2023). They provide a multitude of vital ecosystem services, including nursery habitats for juvenile fish, refuge and sustenance for invertebrates, sediment stabilisation, coastal erosion protection, carbon sequestration, nutrient cycling and mitigation of ocean acidification (Duarte 2000; Bergstrom et al. 2019). Furthermore, these environments, foster protected, endangered and economically important species and account for around 20% of the world's largest fisheries (Unsworth et al. 2019). Comparisons of seagrass beds and neighbouring unvegetated regions consistently revealed distinct community assemblages, featuring greater diversity and complexity of species interactions within the seagrass habitat, attributable to elevated structural complexity (Barnes and Barnes 2012; Park and Kwak 2018; Olesen et al. 2024).

Along the Black Sea coast, seagrass beds encompass 916.9 hectares and represent highly biodiverse habitats (Surugiu et al. 2021), sustaining a large number of macrobenthic invertebrates and fish species, including economically important species, such as sprat, horse mackerel, mullet and anchovy (reviewed in Milchakova 1999). The coast off Burgas and Sarafovo is a particularly important seagrass meadow area, representing 52% of the total seagrass beds area in Bulgaria (Berov et al. 2022).

Foros Bay is a shallow, morphologically complex embayment within the innermost sector of Burgas Bay, the largest bay along the Bulgarian Black Sea coast. The area comprises low-lying coastal plains, sandy barriers and sheltered lagoon-type environments, supporting a heterogeneous mosaic of habitats including infra-littoral sands and muddy sands. Both persistent seagrass meadows and unvegetated seabed areas, some previously occupied by seagrass, are present, providing a natural gradient for assessing biodiversity gains and losses.

Designated under Natura 2000 for its high conservation value, Foros Bay is recognised as a regional biodiversity hotspot (Valchev et al. 2024). Long-term monitoring data indicate that the predominant seagrass species in the region is *Zostera noltei* (Dencheva and Hineva 2016, 2017, 2018, 2021). *Zostera* meadows support a diverse array of metazoan species from various taxonomic groups, including bivalves (*Tellina tenuis*, *Loripes lacteus*, *Lucinella divaricata*, *Solen marginatus*, *Abra ovate*), gastropods (*Rissoa parva*, *Bittium reticulate*), crustaceans (*Upogebia pusilla*, *Carcinus aestuari*, *Polybius vernalis*, *Rhithropanopeus harrisi*, *Palaemon adspersus*, *P. elegans*) and fish species (*Zosterisessor ophiocephalus*, *Nerophis ophidion*, *Hippocampus guttulatus*) (Todorova 2015).

Over the past century, urban expansion, industrial development and nutrient enrichment have increased anthropogenic pressures, reducing light availability, water quality and the natural recovery of seagrass meadows. These cumulative impacts, including the expansion of Burgas City (~ 200,000 inhabitants), the operation of a major oil refinery and the adjacent commercial port, contribute to elevated nutrient and contaminant loads that constrain biodiversity and ecosystem functioning. This establishes critical thresholds that make Foros Bay an ideal system for testing phased, process-based Nature-based Solution interventions aimed at enhancing ecological resilience and maintaining adaptive flexibility under evolving environmental conditions.

Biodiversity assessment in marine nearshore habitats is fundamental for the effective management and conservation of marine resources, especially in regions facing increased anthropogenic pressure. Considering the latter, nearshore habitats, such as seagrass meadows, are amongst the most vulnerable components of the coastal systems, highly susceptible to coastline alterations, habitat modification, eutrophication, pollution and climate-related impacts. Conventional survey methodologies generally fail to accurately capture the full scope of community composition as well as the spatial variability in species composition and diversity within the seagrass ecosystems (Mwamburi et al. 2023). Moreover, conventional methods are often limited by logistical constraints and are generally restricted to specific species or developmental stages. In this respect, eDNA metabarcoding has emerged as a powerful and non-invasive technique for biodiversity assessment that may efficiently overcome these limitations (Compson et al. 2020).

eDNA enables the simultaneous identification of many taxa from water or sediment samples by DNA released into the environment through biological processes, such as tissue shedding, excretion and decomposition, bypassing the necessity for direct organism capture (Rourke et al. 2022; Jo 2023). In addition, eDNA exhibits exceptional sensitivity, rendering it an effective tool for detecting rare and elusive organisms or those present in low abundance. Further, it can be utilised for detecting early life stages, such as larvae or eggs, which are usually difficult to identify through conventional morphological approaches (Rourke et al. 2022).

In recent years, eDNA metabarcoding has been progressively used to study seagrass ecosystems, demonstrating its effectiveness in determining community composition and identifying greater biodiversity compared to traditional methods (Alsaffar et al. 2020; Wesselmann et al. 2022; Titioatchasai et al. 2023; Waters et al. 2023). These studies underscore the capability of eDNA to capture the taxonomic diversity of seagrass-associated communities and to discriminate species assemblages between vegetated and unvegetated areas. However, despite increasing global interest, the use of eDNA metabarcoding in semi-enclosed basins and those historically affected by human activities like the Black Sea remains limited. The main reasons are the need to develop specialised sampling strategies, adapted to the Black Sea's distinct physicochemical and hydrodynamic conditions, the variability of temperature and salinity gradients, as well as the absence of extensive regional DNA reference databases (Zhang et al. 2020).

In this pilot study, we deploy eDNA metabarcoding to evaluate metazoan biodiversity in two distinct nearshore environments in Foros Bay, situated along the Bulgarian Black Sea coast. The main objective was to compare species richness and diversity between *Zostera* seagrass meadows and nearby unvegetated sediments, while also assessing the feasibility of eDNA metabarcoding as a method for biodiversity evaluation in Black Sea seagrass ecosystems.

Materials and methods

Sampling

Ten water samples were taken in June 2025 by SCUBA divers using sterile 2-litre bottles. Five samples were obtained directly above *Zostera* beds in nearshore locations and five were collected from neighbouring bare sand areas beyond the seagrass habitat (Fig. 1).

Each water sample underwent filtration using a sterile 0.22 µm Sterivex cartridge filter (Millipore-Sigma, Burlington, MA, USA) using a peristaltic pump until the filter became obstructed. The filters were instantly sealed and stored at −30 °C in the laboratory until subsequent processing.

eDNA metabarcoding analysis

Genomic DNA was extracted from the Sterivex filters using the MO BIO PowerSoil® DNA Isolation Kit (QIAGEN, Germany) in conformity with the manufacturer's protocol. The concentration and purity of DNA were assessed using a

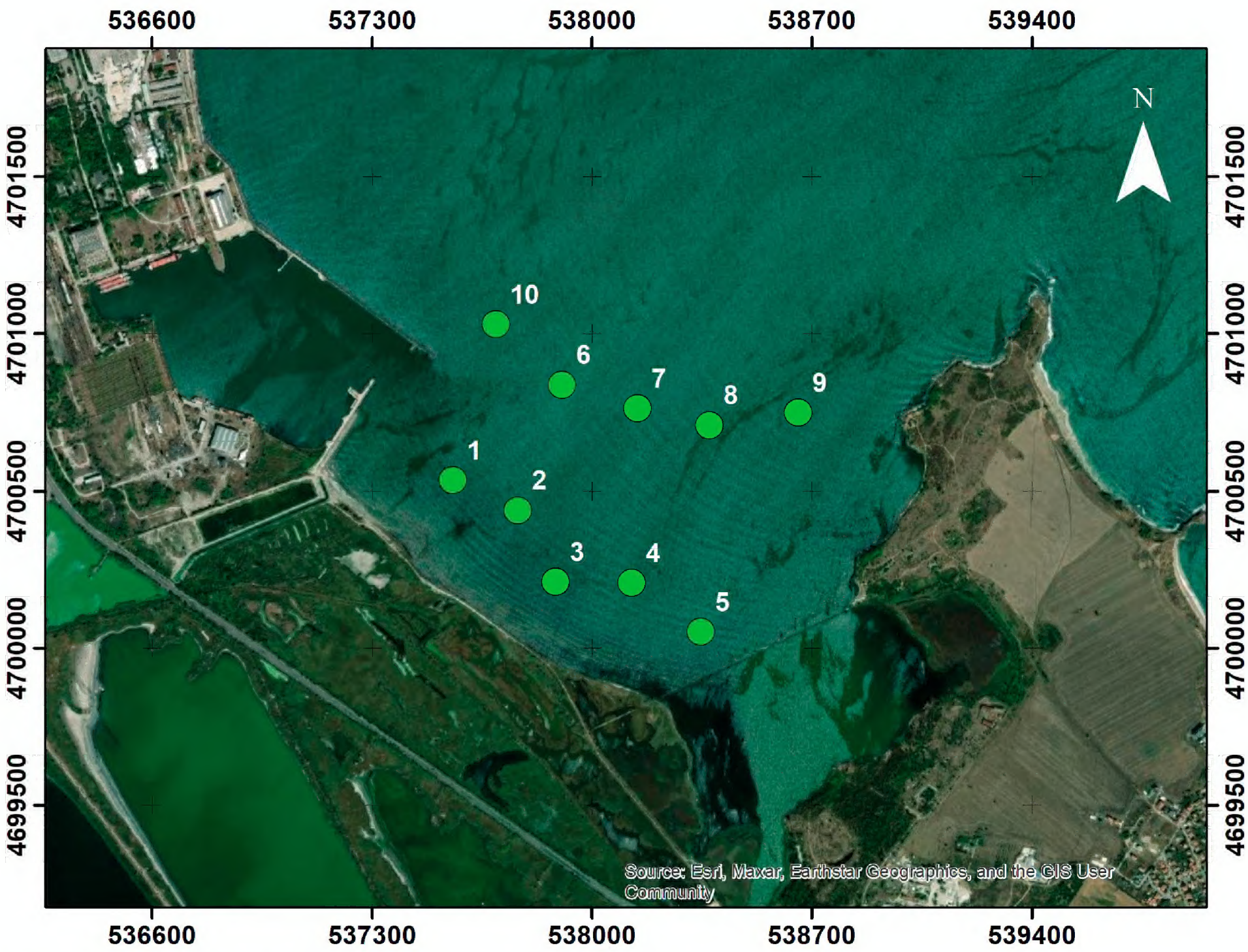

Figure 1. Map of the study area showing sampling station locations.

NanoDrop Lite Plus Spectrophotometer (Thermo Fisher Scientific, USA). Primers targeting the 12S mitochondrial gene region (Miya et al. 2015) and the 18S rDNA V4 region (Stoeck et al. 2010, as modified by Piredda et al. 2017) were used. Libraries preparation and high-throughput of paired-end sequencing were carried out as an external service by Macrogen Inc. (Seoul, South Korea). The sequencing libraries were prepared according to the Illumina Metagenomic Sequencing Library protocols to amplify the target region. The input gDNA 5 ng/10 ng was PCR amplified with 5× reaction buffer, 1 mM of dNTP mix, 500 nM each of the universal F/R PCR primer and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycle condition for 1st PCR was 3 min at 95 °C for heat activation and 25 cycles of 30 sec at 95 °C, 30 sec at 43 °C for 18S rDNA V4 and 50 °C for 12S and 30 sec at 72 °C, followed by a 5-min final extension at 72 °C. The 1st PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA). Following purification, the 10 µl of 1st PCR product was PCR amplified for final library construction containing the index using NexteraXT Indexed Primer. The cycle condition for 2nd PCR was same as the 1st PCR condition, except for 10 cycles. The PCR product was purified with AMPure beads. The final purified product was then quantified using VICTOR Nivo™ (Perkin Elmer) with PicoGreen reagents and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). The libraries were normalised, pooled and quantified using qPCR following the qPCR

Quantification Protocol Guide (KAPA Library Quantification Kits for Illumina Sequencing Platforms) and sequenced using the MiSeq i100 platform (Illumina, San Diego, USA). All the raw sequence data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB107710 (https://www.ebi.ac.uk/ena/browser/view/ PRJEB107710).

Bioinformatics and statistical analyses

Raw demultiplexed sequencing reads underwent quality checks using FastQC v.0.12.1 (Andrews 2010) and primers were removed with Cutadapt v.4.0 (Martin 2011). Amplicon sequence variants (ASVs) were inferred using DADA2 v.1.32.0 (Callahan et al. 2016) within the R programming environment (R Core Team 2025), including quality trimming, error-rate modelling, merging of paired-end reads, chimera removal and ASVs generation.

Taxonomic assignment was carried out using the BLAST+ tool (blastn: v.2.16.0) with a top-hit approach. The PR2 reference sequence database v.5.1.0 (Guillou et al. 2013) was utilised for the 18S data. For the 12S data, a custom database was used, including 637 sequences downloaded from GenBank, representing 117 fish species reported from the Black Sea (Yankova et al. 2014). A similarity threshold of ≥ 97% was utilised for taxonomic assignation. When an ASV matched with equal similarity to two species within the same genus, it was assigned to the genus level. If an ASV matched with equal similarity to several species from different genera, the superior taxonomic rank comprising all matching species was retained. As an additional verification, representative sequences of ASVs matching species not reported from the Black Sea were manually BLAST-searched in the GenBank online database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Alpha-diversity metrics, such as observed taxonomic count (species richness), Shannon–Wiener diversity (H'), Simpson diversity and Pielou's evenness (J'), together with beta-diversity patterns, were computed by utilising the 'vegan' package (Oksanen et al. 2025) in R. Differences in community composition between the two habitat types were evaluated using permutational multivariate analysis of variance (PERMANOVA) inside the 'vegan' package (Oksanen et al. 2025) in R, employing 9,999 permutations. Similarity percentage (SIMPER) analysis was conducted using the 'vegan' package (Oksanen et al. 2025) and Bray-Curtis distances to identify taxa that significantly contribute to the species composition dissimilarity between habitat types. Analysis of indicator species was carried out with the 'indicspecies' R package (De Cáceres and Legendre 2009), to determine which taxa strongly correlated with each habitat. All visualisations were generated using the 'ggplot2' R package (Wickham 2016).

In addition, using the 'betadisper' function in the 'vegan' package, multivariate dispersion analysis was implemented to evaluate the contribution of the habitat type to explain differences in community composition within and between habitats. The Bray-Curtis distance matrices were used to calculate pairwise dissimilarities amongst samples (for both fish and metazoan assemblages). The dispersion differences were tested using a permutation test (9,999 permutations), with lower distances to the respective habitat centroid indicating greater compositional similarity amongst samples (Mushegian 2019; de Celis et al. 2020; Tamashiro et al. 2021).

To address variations in sequencing depth amongst samples, read counts were normalised to relative read abundance by dividing the number of reads attributed to each taxon by the total number of reads in the respective sample, utilising the 'decostand' function (method = "total") inside the vegan package. Binomial generalised linear models (GLMs) were employed to investigate habitat-related variations in taxon detection frequency, determining if taxa were detected more frequently in one habitat type compared to another, independent of read counts. This method utilized taxon presence-absence across samples as the response variable, with habitat type incorporated as a fixed effect in the model to assess variations in detection likelihood between seagrass and bare sand environments (Blabolil et al. 2022; Ruiz-Ramos et al. 2023).

Results and discussion

Metazoan assemblages

A total of 1,011,056 18S V4 reads were acquired by Illumina paired-end sequencing. Quality filtering yielded 768,083 non-chimeric merged reads, which corresponded to 1,448 ASVs. Following taxonomic assignment, 55 ASVs were assigned to Metazoa, representing 48 taxa. In general, metazoan taxa had greater read counts in seagrass stations compared to those from bare sand (Fig. 2).

Species richness and Shannon diversity were moderately higher in seagrass stations (mean SD = 23.40 ± 5.90 and 2.23 ± 0.41, respectively) compared to bare sand stations (17.80 ± 2.49 and 1.70 ± 0.36, respectively) (Fig. 2). Simpson diversity and Pielou's evenness were considerably elevated in seagrass (0.83 ± 0.08 and 0.71 ± 0.09, respectively) compared to bare sand (0.74 ± 0.11 and 0.59 ± 0.12, respectively) (Fig. 3). Nonetheless, none of these disparities attained statistical significance (all p-values > 0.05).

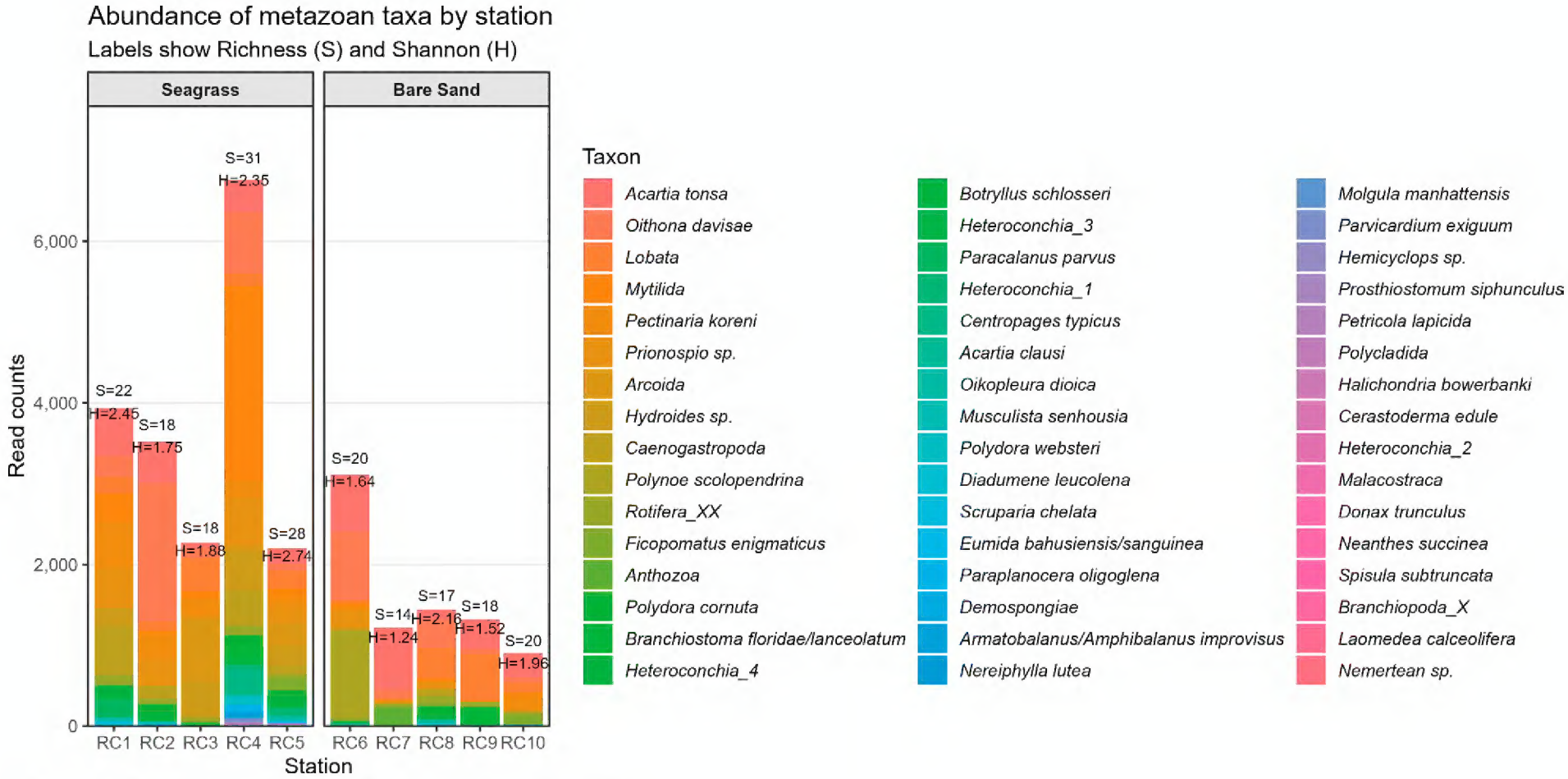

Figure 2. Abundance of metazoan taxa by station.

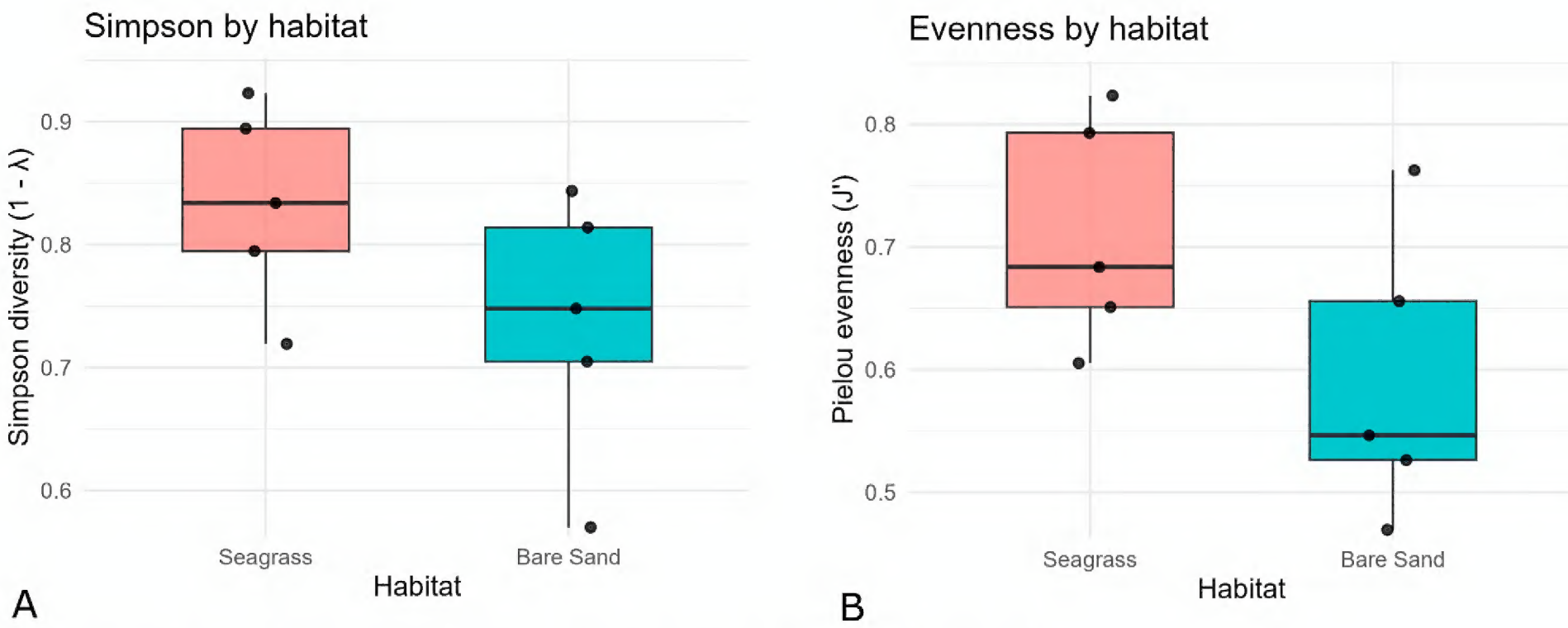

Figure 3. Simpson diversity and Pielou evenness across different habitat types.

The most abundant metazoan taxa in the bare sand sites were *Acartia tonsa* and *Oithona davisae*, representing 28.07% and 17.79% of the total reads combined by habitat, consistently found at all stations. Both copepods have been reported as dominant species within the mesozooplankton community of the Black Sea (Ivanova et al. 2017). The seagrass habitat was dominated by Mytilida and *Oithona davisae* (15.95% and 15.81% of total reads) also detected at all stations (Fig. 4).

Non-metric multidimensional scaling (NMDS), based on Bray-Curtis dissimilarity, revealed partial separation of metazoan communities between seagrass and bare sand habitats (stress = 0.085) (Fig. 5). Permutational multivariate analysis of variance (PERMANOVA) indicated that habitat type had a statistically significant effect on community composition (F = 2.64, R^2 = 0.248, p = 0.0071), explaining approximately 24.8% of the total variation.

SIMPER and indicator species analyses showed that habitat-related variations in metazoan assemblages were predominantly driven by a specific subset of taxa strongly linked to seagrass habitat, rather than an overall restructuring of community composition. The ASVs assigned to Mytiloida_X sp., *Hydroides* sp., *Pectinaria koreni*, Caenogastropoda, Heteroconchia_4, *Botryllus schlosseri* and Heteroconchia_1 were the primary contributors to the Bray-Curtis dissimilarity, accounting for a cumulative contribution of 95% and were identified as an indicator species for the seagrass habitat community structure (IndVal = 0.894–0.998, $p < 0.05$). The latter demonstrated substantially higher abundances in seagrass than in bare sand habitat. On the contrary, *Molgula manhattensis* was the only indicator species associated with bare sand habitat (IndVal = 0.953, p = 0.023).

In alignment with previous studies reporting increased overall macrofaunal abundance, species richness and diversity in seagrass habitats compared to unvegetated sand (Barnes and Barnes 2012; Alsaffar et al. 2020; Olesen et al. 2024), our findings suggest that vegetation enhances the presence and abundance of benthic taxa with pronounced environmental preferences, rather than modifying entire communities. Comparable species-specific alterations in the 18S rRNA communities across bare sand, natural seagrass meadows and transplanted seagrass patches have been recorded in other studies (Titioatchasai et al. 2023).

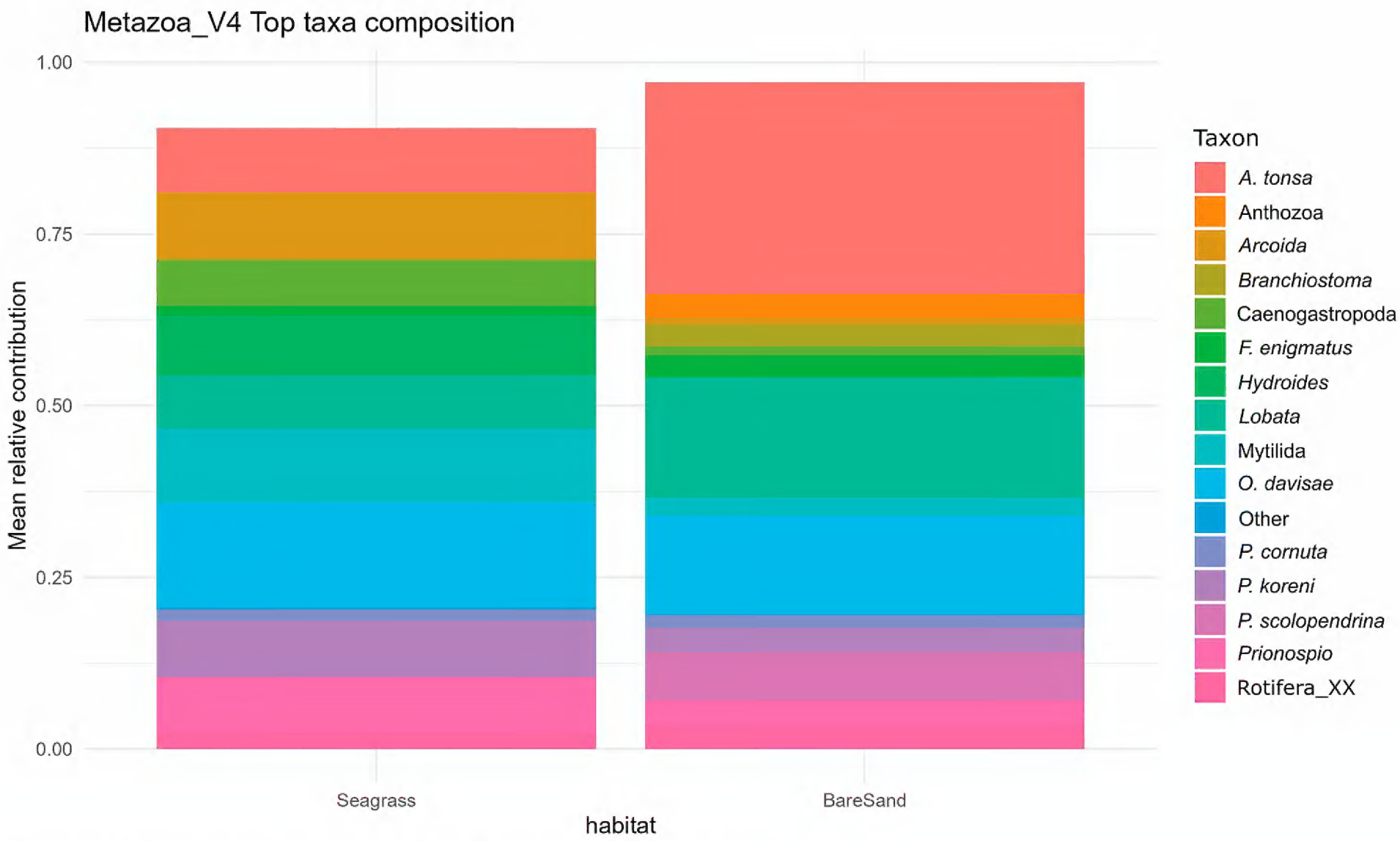

Figure 4. Relative abundance of the dominant metazoan taxa per habitat.

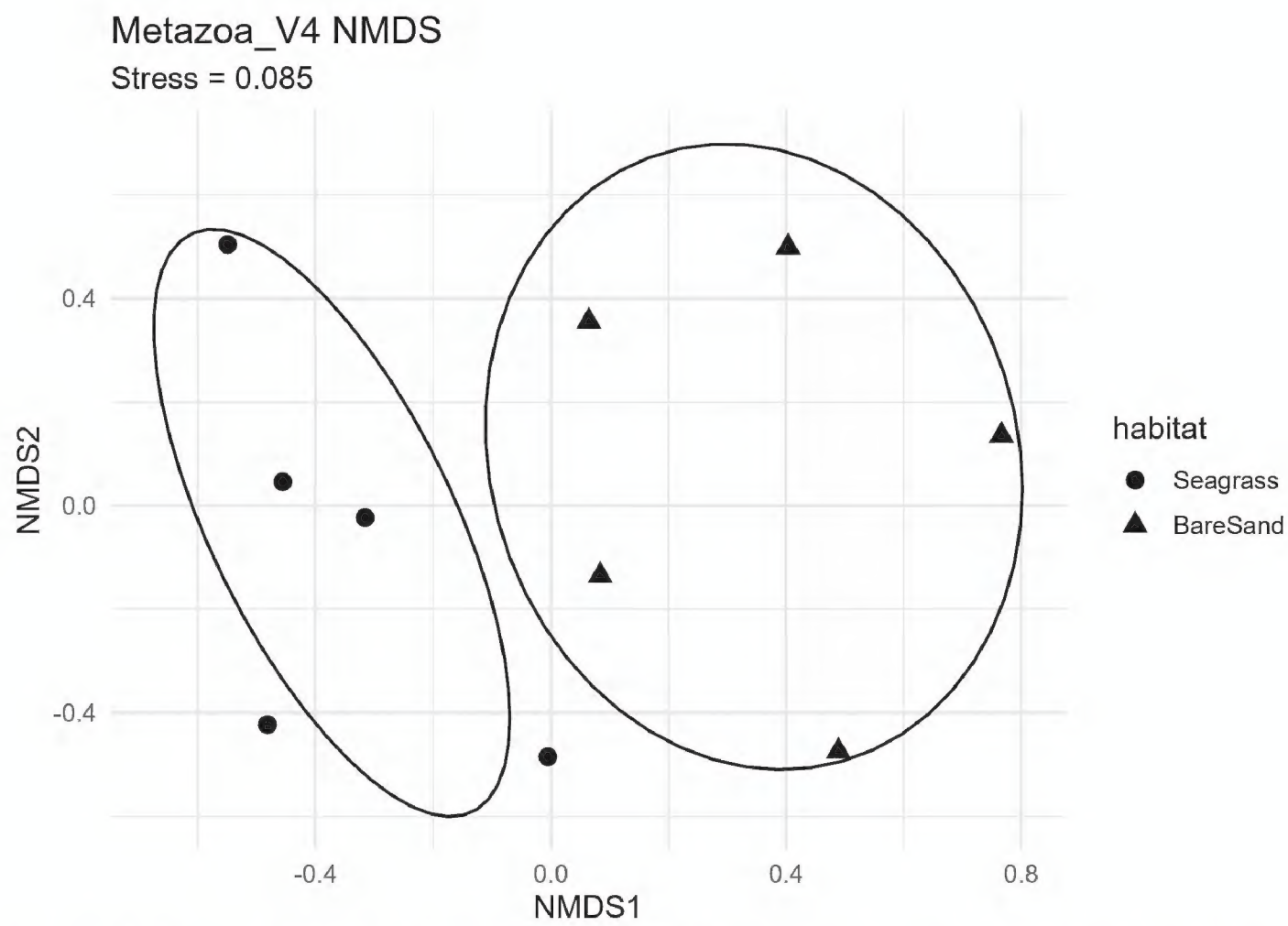

Figure 5. Non-metric multidimensional scaling (NMDS) ordination, based on Bray–Curtis dissimilarity of community composition across seagrass and bare sand habitats.

At the community level, multivariate dispersion analysis revealed no significant differences in within-habitat compositional variability between seagrass and bare sand habitats (betadisper: $F = 0.35$, $p = 0.550$) (Table 1). However, metazoan taxa were detected significantly less frequently in bare sand habitats than in seagrass habitats. Detection probability was approximately 38%

Table 1. Summary of habitat effects on metazoan assemblages. Assumptions were made relating to seagrass as a reference habitat.

Target of analysis	Effect /Response	Model/Test	Habitat effect (Bare sand vs. Seagrass)	Test statistics	*p*-value	Comment
Community variability	Distance to centroid	betadisper (permutation test)	–	$F = 0.352$	0.550	No variation in compositional variability within the habitat
Detection frequency	Occurrence of particular taxa	Binomial GLM	$\beta = -0.479$ ($SE = 0.186$)	$z = -2.58$	0.010*	Reduced detection probability in bare sand habitat
Community Composition	Bray-Curtis dissimilarity	PERMANOVA	–	$F = 2.64$ $R^2 = 0.0071$	0.001**	Significant habitat effect

* Significance level α = 0.05.

lower in bare sand compared with seagrass habitats (binomial GLM: $\beta = -0.479 \pm 0.186$, $z = -2.58$, $p = 0.010$). These results indicate that seagrass habitats support higher detection frequencies of benthic metazoan taxa, while overall patterns of within-habitat variability remain comparable between habitats. The greater structural complexity of seagrass ecosystems presumably enhances the carrying capacity, availability of resources and refuge from predation for benthic invertebrates, enabling their persistence in the area. Small-scale fragmentation and stochastic colonisation may facilitate increased species transfer between habitats, thereby explaining why dispersion remained unchanged (Castro-Fernández et al. 2025).

In seagrass habitats, higher detection frequencies and read counts may indicate that eDNA persists in vegetated regions for extended periods due to reduced water flow and increased particle retention. Metazoan eDNA appears to be more closely associated with local ecological settings than fish eDNA, which is significantly affected by organismal and water movement.

Fish assemblages

A total of 2,839,452 paired-end reads were acquired for the 12S region targeting fish species. Quality filtering yielded 2,467,993 non-chimeric merged reads, which corresponded to 3,651 ASVs. Following taxonomic assignment, 15 ASVs were assigned into fish taxa, encompassing nine species (Fig. 6).

The present study revealed minimal fish diversity in both areas. Four species were exclusively identified in the seagrass stations of Foros Bay: broadnosed pipefish (*Syngnathus typhle*), garfish (*Belone belone*), sand goby (*Pomatoschistus minutus*) and horse mackerel (*Trachurus mediterraneus*). Conversely, the black goby (*Gobius niger*), stellate sturgeon (*Acipenser stellatus*) and chub mackerel (*Scomber colias / Scomber japonicus*) were exclusively identified in neighbouring bare sand environments. Two additional species, the round goby (*Neogobius melanostomus*) and European anchovy (*Engraulis encrasicolus*), were identified in both habitat types.

The distinctive patterns seen in each species indicate that habitat partitioning results from ecological preferences rather than shifts in overall community composition. The occurrence of *S. typhle* and *B. belone*, species often associated with structurally complex environments (Polte and Asmus 2006; Malavasi et al. 2007), highlights the significance of seagrass meadows in offering refuge, sustenance and spawning grounds. Conversely, the occurrence of demersal species, such as *G. niger* and *A. stellatus* in bare sand habitats indicates that

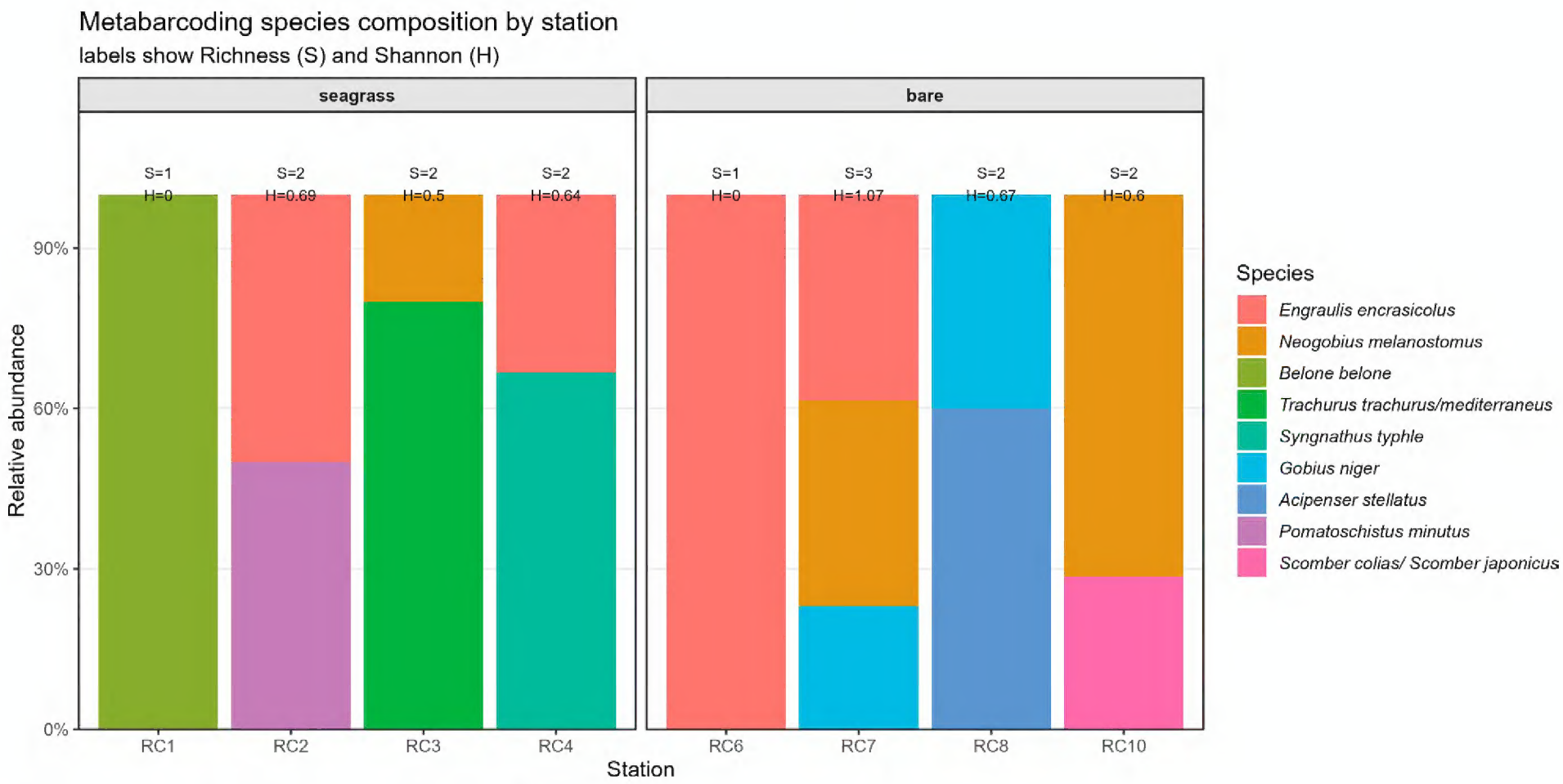

Figure 6. Fish species composition by station.

these species have adapted to their environments in accordance with their life-history and survival strategies.

The presence of commercially important species such as European anchovy, garfish and horse mackerel in seagrass habitats underscores the significance of *Zostera* beds for coastal fisheries from both ecological and economic perspectives. Furthermore, the occurrence of *N. melanostomus* and *E. encrasicolus* in both environments signifies functional connectivity between seagrass and bare sand habitats, highlighting the necessity of preserving habitat heterogeneity in coastal ecosystems to sustain diverse fish assemblages and enhance ecosystem resilience (Alsaffar et al. 2020).

Community-level analyses revealed no significant differences in fish assemblage variability or composition between seagrass and bare sand habitats (Table 2). Multivariate dispersion did not differ between habitats (betadisper: $F = 0.365$, $p = 0.605$), indicating similar levels of within-habitat compositional variability. Likewise, PERMANOVA detected no significant habitat effect on fish community composition ($F = 0.92$, $R^2 = 0.133$, $p = 0.549$). Detection probability of fish taxa also did not differ significantly between habitats (binomial GLM: $\beta = 0.169 \pm 0.581$ *SE*, $z = 0.29$, $p = 0.772$).

These findings suggest that fish assemblages were broadly similar between habitats at the community level, consistent with the high mobility of many coastal fish species and their relatively weak association with benthic habitat type (La Mesa et al. 2011; Lei et al. 2025).

Overall, these results show that, depending on organismal ecology, seagrass ecosystems have a variety of effects on marine populations. Fish assemblages show few habitat-related variations at the community level; nevertheless, at the species level, they exhibit habitat associations, reflecting high mobility and functional connectivity amongst habitat types. Conversely, metazoan assemblages exhibited significant increases in detection frequency and eDNA signal inside seagrass habitats, indicative of heightened habitat dependence and

Table 2. Summary of habitat effects on fish assemblages. Assumptions were made relating to seagrass as a reference habitat.

Target of analysis	Effect /Response	Model/Test	Habitat effect (Bare sand vs. Seagrass)	Test statistics	*p*-value	Comment
Community variability	Distance to centroid	betadisper (permutation test)	–	$F = 0.365$	0.605	No variation in within-habitat compositional variability
Detection frequency	Taxon presence	Binomial GLM	$\beta = +0.169$ ($SE = 0.581$)	$z = 0.29$	0.772	No variation in detection probability across habitats
Community composition	Bray-Curtis dissimilarity	PERMANOVA	–	$F = 0.92$ $R^2 = 0.133$	0.549	No significant habitat effect

* Significance level α = 0.05.

localised population dynamics. Seagrass meadows are important for coastal biodiversity and ecosystem functioning and these findings support the need to combine species-level interpretation with community-level statistics when evaluating habitat effects using eDNA techniques.

From a methodological perspective, while the practical implementation of eDNA metabarcoding as a monitoring tool in the Black Sea is still developing, this pilot study illustrates that eDNA metabarcoding can proficiently characterise and document marine biodiversity in seagrass and adjacent sand habitats. Our findings indicate the effectiveness of eDNA metabarcoding in identifying rare, cryptic and endangered species, which is crucial for sustained biodiversity monitoring and marine resource management. The identification of rare (*S. colias* / *S. japonicus*) and endangered (*A. stellatus*) species at bare sand areas underscores the value of eDNA in uncovering species that may be missed by traditional approaches. Integrating eDNA techniques with conventional sampling methods may provide a more comprehensive insight into fish community dynamics and assist in developing viable conservation and management strategies for coastal environments.

Nonetheless, as with any newly-established genetic technique, constraints, such as inadequate reference databases and restricted taxonomic resolution of the marker region (Ruppert et al. 2019), persist and are evident in the results produced. Numerous ASVs were allocated to various taxa (e.g. *Branchiostoma floridae*/*Branchiostoma lanceolatum*, *Armatobalanus* sp./*Amphibalanus improvises*, *Eumida bahusiensis*/*Eumida sanguinea*) or could solely be classified at a higher taxonomic tier (e.g. Mytilida, Rotifera_XX, Branchiopoda_X). Moreover, species typically inhabiting subtropical to tropical environments, such as *Paraplanocera oligoglena*, were discovered in this study, possibly indicating a deficiency in regional reference data rather than an actual local presence. Consequently, eDNA metabarcoding outcomes must be interpreted judiciously, taking into account both the merits and constraints of the technique.

Standardised protocols for sample collection, laboratory processing and data interpretation must be established to guarantee data comparability and reliability (Çevik and Çevik 2025). The precision and comprehensiveness of these databases, alongside the use of optimal primers, are crucial for reliable species identification, as demonstrated in prior marine metabarcoding research. As sequencing technology advances and database coverage increases, eDNA metabarcoding may evolve into a standardised and efficient approach for ecosystem monitoring (Yang et al. 2024).

Conclusions

Our study indicates that seagrass ecosystems influence marine populations of fish and benthic metazoans in distinct ways. Seagrass does not homogenise community composition or reduce within-habitat diversity at the assessed geographic scale, as multivariate dispersion showed no significant difference between seagrass and bare sand habitats for either taxonomic group.

No habitat-related changes were observed relating to fish assemblage composition, compositional variability and detection frequency. The correlation between fish community structure and benthic habitat type is weak, possibly attributable to fish mobility and their frequent movement between habitats. Species-level patterns indicated habitat connections, revealing that structurally connected species were exclusively found in seagrass, whereas demersal species were confined to bare sand, implying a functional habitat partitioning that community-level metrics fail to capture.

In contrast, metazoan assemblages showed significant habitat-related differences in community composition and detection frequency, with taxa being detected more frequently in seagrass habitats than in bare sand environments. These findings are consistent with ecological theory suggesting that structurally complex seagrass ecosystems can enhance habitat suitability and support greater diversity and persistence of benthic invertebrates.

Our findings highlight the significance of organismal ecology and analytical scale in eDNA-based habitat comparisons. Benthic metazoans and ecologically and economically important fish species succeed in seagrass meadows, which sustain functional connectivity with adjacent bare sand habitats. These findings underscore the necessity of protecting and rehabilitating seagrass meadows as essential components of coastal habitat compositions, as their ecological significance could be demonstrated through increased species diversity and biomass rather than reduced community variability. Consequently, the loss of seagrass habitats could have a disproportionate impact on benthic biodiversity and ecosystem functioning.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualisation: PI, NV. Sampling: BP, NV. Formal analysis: PI, ND, IZ. Methodology: ND, PI, IZ. Software: ND, IZ. Visualisation: IZ, PI, ND. Writing - original draft: PI, ND, IZ, NV. Writing - review and editing: PI, ND, IZ, NV. Funding acquisition: NV. All authors contributed to the final version of the manuscript and approved the submitted version.

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Data availability

All of the data that support the findings of this study are available in the main text.

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